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Methylprednisolone Inhibits Pemphigus Acantholysis in Skin Cultures

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Glucocorticosteroids are used to treat patients with pemphigus, but the mechanism of action is unknown. We studied the effect of methylprednisolone on acantholysis induced in vitro by incubation of normal skin with plasma from a patient with pemphigus. Normal human breast skin was maintained in organ cultures for several days in Ham F-10 medium. Plasma from a patient with active pemphigus vulgaris caused suprabasilar epidermal acantholysis when added to this culture system. In control cultures (F-10 medium and fetal bovine serum), no acantholysis occurred. Acantholysis was prevented when breast skin was preincubated for 24 h in a 0.25 mM solution of methylprednisolone in F-10 medium and fetal bovine serum, suggesting that methylprednisolone directly inhibits acantholysis. No suppression of acantholysis occurred when the methylprenisolone was added to the culture system simultaneously with the pemphigus plasma, suggesting a time requirement for alteration of cellular events. The inhibition of acantholysis was not caused by cell death since methylprednisolone did not alter keratinocyte viability as determined by exclusion of trypan blue dye when keratinocytes were exposed to pemphigus plasma. Similarly, the inhibition of acantholysis was not due to dissolution, alteration, or coating of pemphigus antigen on epidermal cells, since the intercellular antibodies in the plasma bound as well to methylprednisolone-treated epidermis as to untreated epidermis.

Pemphigus is a bullous skin disease in which epidermal cells lose their attachment to each other. Patients with pemphigus have antibodies in their skin and plasma which bind to the intercellular areas of epidermis and may be pathogenic [1]. These antibodies can induce epidermal acantholysis in vitro by a mechanism which requires neither complement nor inflammatory cells [2]. High doses of systemic corticosteroids may prevent the appearance of new bullae in patients with pemphigus [3]. The mechanism by which corticosteroids inhibit acantholysis is unknown. We investigated the effect of methylprednisolone on acantholysis produced by pemphigus antibody in vitro.

METHODS

Effect of Methylprednisolone on Acantholysis

Human skin was maintained in skin organ culture by a modification of the methods of Sarkany et al [4]. Acantholysis was induced in specimens by the method of Schiltz and Michel [5]. Squares of normal human breast skin (4 × 4 × ½ mm) were placed on sterile lens tissue. This tissue paper had waxed edges so that it and the skin specimen would float in various test media. Tissue, tissue paper, and media were placed in a watch glass and maintained in a sterile, covered culture dish containing wet gauze to insure high humidity. The system was incubated in a humidifed 4% CO_2 atmosphere at 37°C. Specimens were incubated for 3 days. After incubation, organ culture specimens were removed from their paper rafts, fixed in formalin, sectioned, and stained with hematoxylin and eosin. Specimens were then coded and examined by light microscopy to determine whether acantholysis was present. Acantholysis was defined as the presence of a suprabasilar cleft. Specimens were read blindly by 3 histopathologists to eliminate any possible prejudice.

To study the effects of methylprednisolone on inhibition of acantholysis, skin specimens were incubated in Ham F-10 medium [6] (1 ml) and fetal calf serum (FCS) (1 ml) with methylprednisolone (0.25 mM) (Upjohn Co., Kalamazoo, Michigan). Methylprednisolone at a 0.25 mM concentration approximates the distribution of 1 g of methylprednisolone in the extracellular fluid of a 60-kg woman. After a variable preincubation period, citrated plasma (1 ml) derived from a patient with active pemphigus was added to the medium (Table I). All experiments were repeated at least 4 times. To induce acantholysis, plasma from a patient with active pemphigus with intercellular antibodies at 1:320 titer was added to the medium either initially or after a 24-h preincubation in FCS and Ham F-10 medium. As controls, organ culture specimens were incubated in Ham F-10 medium containing either FCS or normal human plasma either with or without 0.25% mM methylprednisolone (Table II).

Effect of Methylprednisolone on Antibody Binding

Two experiments were performed to study the effect of methylprednisolone on the binding of pemphigus antibody to the intercellular areas of human epidermis. To determine whether methylprednisolone absolutely inhibited pemphigus antibody binding, human skin was incubated in organ cultures as described above in medium initially containing FCS (1 cc), Ham F-10 medium (1 cc), and 0.25 mM methylprednisolone. After 24-h preincubation, pemphigus plasma (1 cc) was added. Two days later, specimens were removed from medium and frozen in liquid nitrogen. Four-micron frozen tissue sections were studied for the presence of IgG using a standard direct immunofluorescence technique [7].

To determine whether methylprednisolone partially inhibited the binding of pemphigus antibody to the intercellular epidermis, studies were conducted to see whether preincubation of skin with methylprednisolone could reduce the apparent titer of pemphigus antibody when the incubated skin was subsequently used as a substrate for indirect immunofluorescence. Human skin was incubated in organ cultures as described above. Specimens were incubated for 24 h in equal amounts of Ham F-10 medium and FCS with or without 0.25 mM methylprednisolone. Following incubation, specimens were frozen, sectioned, washed, and incubated for 30 min in serial dilutions of pemphigus plasma with or without 0.25 mM methylprednisolone. Specimens were washed in phosphate-buffered saline and incubated with fluorescein-labeled goat antihuman IgG for 30 min. After washing, specimens were examined for fluorescence of the intercellular areas of epidermis.

Effect of Methylprednisolone on Cell Viability

Cell viability was determined in explants themselves. Fresh human skin was incubated with and without methylprednisolone and with pemphigus or control plasma as described above. After 1-, 2-, or 3-day incubation, the epidermis was separated from the dermis by incubation in 0.5% trypsin-EDTA solution for 1 h at 37°C. The epidermal sheet was cut into squares and teased apart in a solution of DNAase 0.025%. The epidermal sheets were titurated briskly in and out of a small pipette to dislodge keratinocytes. Two parts of suspension were mixed with 1 part of 1% trypan blue solution and examined after 5 min for the ability of cells to exclude trypan blue dye.

RESULTS

Effect of Methylprednisolone on Acantholysis

Acantholysis was recorded as present if suprabasilar intraepidermal clefting was present in skin specimens. Acantholysis

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Abbreviations:
FCS: fetal calf serum

was present after incubation in control solutions containing pemphigus plasma (Fig 1). No acantholysis was observed in controls utilizing normal plasma (Table II). Acantholysis was always absent in skin maintained in medium containing pemphigus plasma and methylprednisolone after a 24-h preincubation in methylprednisolone (Fig 2). After a preincubation of only 1 or 4 h in methylprednisolone, separation and acantholysis were induced by pemphigus plasma. In these equivocal specimens, some separation of cells in the suprabasilar area suggested early acantholysis, but free-floating epidermal cells were absent. The same equivocal changes were observed each time the experiment was repeated using the short 1- to 4-h preincubation.

TABLE I. *Effect of preincubation of skin in methylprednisolone on elicitation of acantholysis*

Time of addition of pemphigus plasma (PP) to initial medium[a]				
0 hours (day 1)	1 hour	4 hours	24 hours (day 2)	Acantholysis day 4
PP (1 ml)	—	—	—	Present
—	PP (1 ml)	—	—	± Present
—	—	PP (1 ml)	—	± Present
—	—	—	PP (1 ml)	Absent

[a] The initial medium consisted of fetal calf serum 1 ml, F-10 medium 1 ml, methylprednisolone 0.25 mM.

TABLE II. *Control experiments*

Experiment no.	Time of addition of reagents to skin culture			Acantholysis at 72 hours
	0 h	24 h	48 h	
1	FCS 1 ml F-10 1 ml	PP 1 ml	–	Present
2	FCS 1 ml F-10 1 ml	–	PP 1 ml	Present
3	FCS 1 ml F-10 1 ml	NP 1 ml	–	Absent
4	FCS 1 ml F-10 1 ml	NP 1 ml MP	–	Absent

Schedule of control experiments which show that pemphigus plasma causes acantholysis but normal plasma and methylprednisolone do not. Skin cultures were incubated in fetal calf serum and Ham F-10 medium. Pemphigus plasma, normal plasma, or methylprednisolone were added 24 or 48 h later.

FCS = fetal calf serum; F-10 = Ham serum F-10 medium; PP = plasma from pemphigus patient; NP = plasma from normal control; MP = methylprednisolone (40 mg/ml).

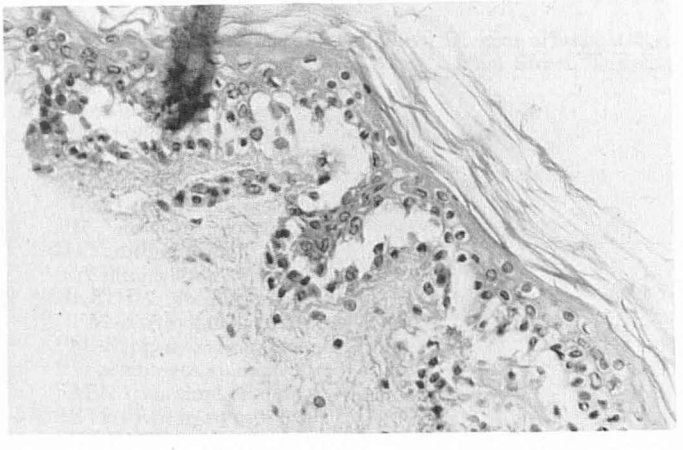

FIG 1. Photomicrograph of skin showing acantholysis after incubation with media for 24 h and pemphigus plasma for 2 days (original magnification ×100).

FIG 2. Photomicrograph of skin showing inhibition of acantholysis by methylprednisolone. The skin had been incubated with 0.25 mM methylprednisolone for 24 h and with methylprednisolone and pemphigus plasma for 2 more days (original magnification ×100).

Effect of Methylprednisolone on Antibody Binding

Immunofluorescence studies demonstrated that methylprednisolone neither abolished nor inhibited binding of pemphigus antibody to the intercellular epidermis. The pattern of antibody binding was typical of that seen in skin of patients with pemphigus vulgaris. When skin and skin sections incubated with methylprednisolone were used as a substrate for indirect immunofluorescence, there was no reduction in the apparent titer of intercellular antibody.

Effect of Methylprednisolone on Cell Viability

After 1 to 3 days in organ culture, the viability of cells incubated with pemphigus plasma with or without methylprednisolone was about 10%. The viability of cells incubated with normal human plasma with or without methylprednisolone was about 60%. In other words, methylprednisolone had no effect on cell viability, while pemphigus plasma appeared cytotoxic. This cytotoxicity occurred during the first 24 h of incubation with pemphigus plasma. No additional cytotoxicity was observed after 48- or 72-h incubations.

CONCLUSIONS

Pemphigus vulgaris is a chronic bullous skin disease which may cause death. Systemic glucocorticosteroids can prevent the development of new bullae, and may be life-saving. Large doses of systemic corticosteroids as high as 1 g of prednisone daily are required [3]. This is much greater than the daily doses of corticosteroids necessary to completely suppress inflammation in inflammatory disorders such as lupus erythematosus and rheumatoid arthritis. This suggests that glucocorticoids suppress bulla formation by a mechanism unrelated to inflammation.

Binding of antibody to epidermal cells may induce them to secrete a nonlysozomal protease which alters the intercellular cement substance to cause epidermal dyshesion [2,8,9]. Such a mechanism does not require the participation of complement, but does require epidermal cell viability and is therefore consistent with requirements for acantholysis in vitro. We have found that methylprednisolone in high concentrations can completely inhibit acantholysis produced by pemphigus antibody in organ culture. Just how methylprednisolone does this is not known. We observed that methylprednisolone does not affect antibody binding. We also found no glucocorticoid effect on cell viability. A similar absence of toxicity from corticosteroids had been made by others using hepatocytes [10] and myocardium [11] in vitro. Perhaps methylprednisolone directly inhibits its synthesis or release of protease by epidermal cells.

Schiltz and Michel were unable to block in vitro pemphigus acantholysis with glucocorticosteroids [9]. We also found no inhibition unless the organ culture was preincubated in methylprednisolone for 4–24 h before addition of pemphigus antibodies. However, with adequate preincubation, acantholysis was totally suppressed. Perhaps a 4- to 24-h lag time is required for methylprednisolone to diffuse to the epidermal cells, enter them, and inhibit metabolic events. In vitro, prednisone may also inhibit antibody synthesis although the major decline in antibody titers occurs 3–4 weeks after glucocorticoid administration [12–14].

In order to suppress acantholysis in organ culture, high concentrations of methylprednisolone are required. Complete inhibition of acantholysis was observed only when the incubation medium contained methylprednisolone in a concentration of at least 0.25 mM [15]. Although concentrations of this magnitude have been approached physiologically during treatment of patients with active pemphigus, this concentration requirement is higher than that usually required to control the disease. Perhaps a longer preincubation of skin with lower concentrations of methylprednisolone would also inhibit acantholysis in tissue culture, but artifacts occur in skin cultures after 72 h [2] which make interpretation difficult. Alternatively, the events in tissue culture may not absolutely reflect events in patients, so concentration requirements may not be directly comparable.

Schiltz et al [2] observed that pemphigus antibody was cytotoxic to epidermal cells in culture. The severity of cytotoxicity appeared to directly correlate with the titer of antibody. In our culture system, pemphigus antibody also caused keratinocytes to die. The clinical relevance of this cytotoxicity, if any, is uncertain. Methylprednisolone neither caused cell death, nor prevented the cytotoxicity of pemphigus plasma.

Our studies support the concept that acantholysis of pemphigus is caused by a binding of antibody to epidermal cells. Methylprednisolone in organ culture can block acantholysis. The high dose requirements are consistent with the clinical observation that high doses of systemic corticosteroids are necessary to successfully stop new blister formation in patients with pemphigus.

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